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Can arbuscular mycorrhizal fungi be used to control the undesirable grass *Poa annua* on golf courses?

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Summary

1. *Poa annua* (annual meadow-grass or annual bluegrass) is the most problematic weed of temperate zone golf putting greens. In the UK there are no chemicals approved for its control, although several herbicides and plant growth regulators are available in the USA. Reducing *P. annua* levels in fine turf would greatly reduce the heavy reliance on pesticides and water that currently exists.

2. This paper reports on an observational and a manipulative study in golf putting greens, aimed at determining whether arbuscular mycorrhizal (AM) fungi have any potential for the reduction of this weed in fine turf.

3. All 18 greens on three golf courses were sampled, and in two courses a negative relation between AM fungi and *P. annua* abundance was found, upholding previous results. In greens where AM fungi were relatively common (as measured by root colonization), *P. annua* was rare, and vice versa. Furthermore, when the fungi were common, abundance of the desirable turfgrass *Agrostis stolonifera* was greater.

4. Two explanations are suggested for these relations, a competitive one, in which AM fungi alter the balance of competition between the two grasses, and an antagonistic one, in which the fungi may directly reduce the growth of *P. annua*.

5. In a manipulative experiment, where mycorrhizal inoculum was added to a golf green, the colonization level of *A. stolonifera* roots was enhanced, as was the abundance of this grass. Furthermore, there was a suggestion that adding inoculum could decrease the abundance of *P. annua*.

6. AM fungi have the potential to be a much more environmentally sound method of *P. annua* control in sports turf than the currently used chemicals.

Key-words: Agrostis stolonifera, arbuscular mycorrhiza, plant competition, turfgrass.

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Introduction

The production and maintenance of high-quality fine turf surfaces for use on golf putting greens is a costly and labour-intensive business (Adams & Gibbs 1994). In the UK, golf greens are sown with a seed mixture (approximately 80 : 20 by weight) containing species of bentgrass (*Agrostis*) and fescue (*Festuca* spp.). The most widely used species are *Agrostis stolonifera* L. and *A. capillaris* L. and various subspecies of *Festuca rubra* L. When mown at a height of 5 mm or less, the bentgrass/fescue mixture can produce fast, true and firm surfaces, with a uniform grass cover and suitability for all-year play, which are the desired characteristics of a high-quality putting green (Hayes 1990).

However, a major problem with the majority of putting greens is that they become invaded by the weed grass *Poa annua* L. (annual meadow grass or annual bluegrass). This problem is not confined to the UK, but occurs in all other temperate areas of the world, such as the USA (Wu, Till-Bottraud & Torres 1987) and Australia (Lush 1989). *Poa annua* is most characteristic of disturbed habitats, but it is able to persist even in long-term stable grassland habitats, if these are subject to heavy trampling and intense defoliation (Hutchinson & Seymour 1982). Golf putting greens are particularly subject to these factors; a well-used course may experience at least 100 000 rounds per year and in the growing season the greens are mown daily to a height of about

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4 mm. Cutting occurs throughout the year, but with reduced frequency in winter (Perris & Evans 1996). As a result, bentgrass/fescue greens rapidly become dominated by this weed, and in many cases the green eventually becomes a *P. annua* sward, with the desirable grasses dying out. The sward then becomes self-regenerating, because *P. annua* can flower and set seed at a height of less than 5 mm (Hutchinson & Seymour 1982) and new plants arise from the seed bank which is dominated by this species (Lush 1988). Addition of *A. stolonifera* seed is a commonly used method of attempting to maintain the abundance of this grass, and thereby resisting invasion by *P. annua* (Perris & Evans 1996).

Poa annua is generally considered to be undesirable in putting greens because its shallow root system makes it particularly susceptible to abiotic stress, especially water availability (Adams & Gibbs 1994). This is important, because water use is an expensive and often controversial aspect of golf course management (Kneebone, Kopec & Mancino 1992). *Poa annua* is also susceptible to a number of turfgrass diseases (Fermanian *et al.* 1997), especially *Fusarium* patch [causative organism *Microdochium nivale* (Fr.) Samuels & I.C. Hallett; Adams & Gibbs (1994)]. In a recent survey of pesticide usage on golf turf, it was found that 84% of all fungicide application is directed at *Fusarium* patch (A.C. Gange, unpublished data). Furthermore, the growth habit of *P. annua* is different to that of bentgrasses and fescues, meaning that a green which contains patches of each (the normal situation) will have an irregular putting surface. The livelihood of a golf club may depend on the quality of its greens, and therefore the amount of *P. annua* in them. In a few clubs in the UK the problem is regarded as serious enough for green staff to be assigned the task of hand-weeding it from the turf.

A number of more conventional methods have been investigated for the control of *P. annua* in fine turf. In the USA, several herbicides have been used with varying success (Johnson 1975, 1982; Callahan & McDonald 1992). More recently, plant growth regulators have been tried, notably paclobutrazol and flurprimidol (Johnson & Murphy 1995, 1996), with the former being the most effective. No chemicals are approved for use against *P. annua* in the UK, however. In addition, application of pesticides can be costly and may present a problem of groundwater pollution if the green is well irrigated (Petrovic 1994). Of no less concern is the fact that their use may represent a health risk to players and greens staff (Moon, Shin & Lee 1994). Therefore, it is timely to seek an alternative, more natural, approach to the control of this weed.

Recently, the possibility of using microbial control methods have been investigated. Zhou & Neal (1995), Imaizumi *et al.* (1997) and Imaizumi, Tateno & Fujimori (1998) have reported varying amounts of *P. annua* control with the bacterium *Xanthomonas campestris* pv. *poae*. Egli & Schmidt, and Horwath, Elliott & Lynch (1998) have found that an isolate of the rhizobacterium *Pseudomonas putida* (Trevisan) Migula can be effective in some situations. Meanwhile, Gange (1994) reported that the abundance of *P. annua* in greens on one course was negatively related to the amount of arbuscular mycorrhizal (AM) fungi in the soil. When the fungi were very low in abundance *P. annua* was common, and vice versa. The converse was true for *A. stolonifera* in that higher levels of AM colonization appeared to be related to greater abundance of this grass.

There have been very few reports of the occurrence of mycorrhizal fungi in turfgrass, notable exceptions being the work of Koske, Gemma & Jackson (1997a,b). In these studies, a number of AM fungal species were found as spores in turfgrass soil, including areas dominated by *P. annua*. This may appear surprising at first, as Hutchinson & Seymour (1982) state that no mycorrhizal associations were found with this grass, while Harley & Harley (1987) consider it as weakly mycorrhizal, if at all. However, Gange (1994) reported colonization levels as high as 12% RLC (root length colonized), and colonization has also been found in more natural plant communities (Gange, Brown & Farmer 1990). Therefore, AM fungi do exist in turfgrass soil, but whether they play any role in determining the abundance of species in the grass community is unknown. In natural plant communities, these fungi can affect plant community structure by enhancing growth of the more strongly mycorrhizal plant species (Gange, Brown & Sinclair 1993; van der Heijden *et al.* 1998a). If they can deter the dominance of *P. annua* while promoting the growth of bentgrass (Gemma *et al.* 1997a) then the possibility exists that these fungi could be used as an alternative to chemicals for the control of *P. annua* in fine turf. Therefore, our aims were to examine whether the negative relation between mycorrhizas and *P. annua* is a universal one, and to add mycorrhizal inoculum to a working putting green to see if colonization levels could be altered and grass abundance changed. In the latter situation, mycorrhizal addition was compared with the traditional practice of *Agrostis* overseeding.

Materials and methods

COURSE SPECIFICATIONS

Three 18-hole courses were selected for this study. As the livelihood of a golf club depends on the quality of its greens, and the amount of *P. annua* in the greens is a sensitive subject, the identity of the clubs cannot be revealed. They are referred to hereafter as

courses A, B and C. All three courses were of similar age, being opened in 1922, 1928 and 1923, respectively, and all were in the county of Surrey, UK. All greens on each course were soil-based, constructed from acidic sandy loam, overlying the Bagshot Sands. They were originally sown with bentgrass (*A. stolonifera*)/fescue (*Festuca* spp.) mixtures but now consist of *P. annua* with some *A. stolonifera* and occasional patches of *Festuca* spp. The pH range in the greens was: course A, 4·7–6·8; course B, 4·5–7·1; course C, 4·3–6·5. Greens were mown daily in the summer growing season, less frequently in winter, to a height of between 4 and 5 mm. All greens were equipped with automatic irrigation systems and were watered as necessary during dry spells. Fertilizer and top dressing application was similar on each course and in line with the recommended requirements for these courses (Adams & Gibbs 1994).

OBSERVATIONAL SAMPLING

Course A was sampled during July 1996 and courses B and C during July 1997. On each course, three replicate soil cores, each measuring 2·5 cm in diameter and 5 cm deep, were taken from random positions on every green. Each core was hand sorted under a dissecting microscope and the tillers of each grass species separated and counted. These were then converted to numbers per m^2. Tillers of *Festuca* spp. were too sporadic for mycorrhizal sampling to be performed. Therefore roots of *P. annua* and *A. stolonifera* tillers were washed and combined, to produce a composite sample of each species from each core. A number of root fragments, each measuring about 1 cm, were taken randomly from each species sample and placed on a microscope slide. These were examined at ×100 using a Zeiss Axiophott epifluorescence microscope fitted with a UV lamp. The filters used were as described by Merryweather & Fitter (1991), giving a transmission of 455–490 nm blue. Under these conditions, the arbuscules (the only definitive structure of the mycorrhiza) can be recorded because they autofluoresce (Ames, Ingham & Reid 1982). Colonization levels of arbuscules were assessed using the crosshair eyepiece intersection method of McGonigle *et al.* (1990). Approximately 150 intersections per slide were recorded, to give a measure of percentage root length colonized (%RLC).

STATISTICAL ANALYSIS

Differences between greens in grass tiller counts, the proportion of a grass species in the sward, and mycorrhizal colonization were examined using Kruskal–Wallis one-way analysis of variance. This test was employed throughout because not all the data sets satisfied the assumptions of normality, and the replicate number (three cores per green) was low. Replicate numbers could not be higher, for fear of damaging the putting surfaces. Subsequent separation of means was performed with a Tukey-type multiple comparison (Zar 1996). Relations between the abundance of *P. annua* and its mycorrhizal colonization were examined with linear regression. All percentage data were subjected to the angular transformation prior to analysis. All analyses were performed with the UNISTAT statistical package (version 4.0).

MANIPULATIVE EXPERIMENT

A practice putting green at course A was used for this experiment. The green is managed in a similar way to the main course, in terms of mowing, fertilizer, top dressing and water application. However, it does not receive as much compaction as the main greens, being walked on by fewer people. It contained *P. annua*, *A. stolonifera* and *Festuca* spp. Bicarbonate-extractable P concentration was $22 \pm 4{\cdot}6\ \mu g\ g^{-1}$. In May 1997, 20 $2{\cdot}5 \times 5$-cm soil cores were taken from random positions in the green. Spores of AM fungi were extracted from these by wet sieving and sucrose centrifugation (Brundrett *et al.* 1996). Identification of AM taxa was performed by comparison with authenticated specimens and reference to Yao, Pegler & Young (1996).

Twenty-four plots, each $0{\cdot}5 \times 0{\cdot}5$ m and separated by 2 m, were marked out in a randomized block design, with four plots per block. In each block one plot was allocated randomly to one of four treatments: (i) addition of *A. stolonifera* seed at the rate of 4 g per plot (16 g m^{-2}); (ii) addition of AM inoculum (Vaminoc-T; MicroBio Ltd, Hemel Hempstead, UK) at the rate of 5 g per plot (20 g m^{-2}); (iii) addition of seed and inoculum; and (iv) addition of neither (control). Infectivity of the inoculum was 863 ± 71 propagules g^{-1}, giving an application rate of 17 260 propagules m^{-2}. The treatments were applied once, in early July 1997, and immediately after application the sprinkler system was turned on to irrigate the green for half an hour. A week before the first sample, and then at approximately 6-week intervals thereafter, grass tiller abundance in the plots was sampled non-destructively with the point quadrat method (Laycock & Canaway 1980). On each occasion, a linear steel grid containing 10 3-mm point quadrat pins was placed diagonally across each plot, with the central spike of the grid in the centre of each plot. The grid was placed twice in each plot, each sample being at right angles to the other. The total touches of each grass species was counted on each pin and the values for the 20 pins summed to give a value for the plot. On the first

and last grass samples (June 1997 and May 1998), one 2·5 × 5-cm core was taken from each plot and the roots prepared for mycorrhizal recording as described above. The hole left by each core was filled with sterile top dressing (a mixture of 80% sand and 20% fen loam). Core samples could not be taken on every sampling date because of destruction of the putting surface.

STATISTICAL ANALYSIS

All data were square-root transformed, to meet the assumptions of normality and homogeneity of variances. The total tiller count per plot for each grass species was analysed on the first date with a two-way ANOVA, employing block, seed addition and AM fungal addition as main effects. This was to ensure that plots were homogeneous at the start of the experiment, because *P. annua* does tend to occur in discrete patches on a golf green. Tiller counts of all grass, *P. annua*, *A. stolonifera* and *Festuca* spp. were then analysed over the experimental period with a repeated measures ANOVA, employing block, seed and fungal addition and date as main effects. To test whether the treatments had altered AM colonization of grass roots over the experimental period, mycorrhizal colonization of the three grass species was compared on the first and last sample dates with the Wilcoxon paired-sample test (Zar 1996).

Results

OBSERVATIONAL SAMPLING

The total tiller count varied dramatically between courses, with course A having the highest counts and course C the lowest (Fig. 1). However, within courses, the greens were remarkably similar in their total cover. Although there was some evidence of intergreen differences (course A, $\chi^2 = 27{\cdot}8$, $P = 0{\cdot}047$; course B, $\chi^2 = 26{\cdot}2$, $P = 0{\cdot}071$; course C, $\chi^2 = 29{\cdot}7$, $P = 0{\cdot}029$; all d.f. = 17), these were not significant using the Tukey test. Given that within each course 153 multiple comparisons were made, and one would expect about 5% (eight) of these to be significant by chance alone, it is safe to assume that the greens on each course did not differ in their total sward cover.

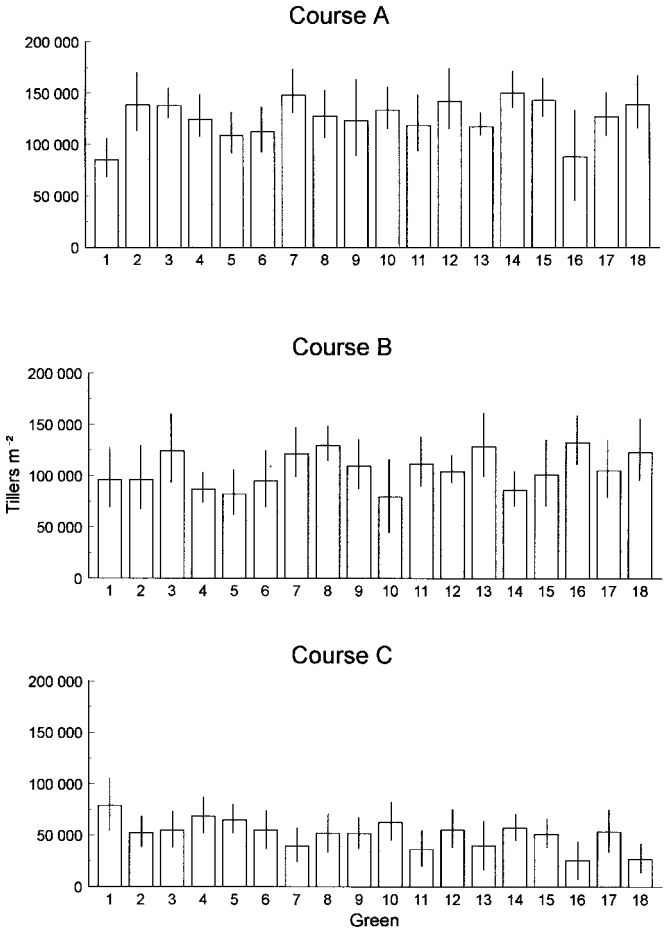

Fig. 1. Mean total grass tiller count m^{-2} for all 18 greens on the three golf courses. Lines represent one standard error.

All greens on each course were dominated by *P. annua*, which in many cases formed 100% of the sward (particularly course B) (Fig. 2). However, this proportion was not consistent within courses (course A, $\chi^2 = 49{\cdot}33$, $P < 0{\cdot}001$; course C, $\chi^2 = 49{\cdot}67$, $P < 0{\cdot}001$). Course B was not analysed because virtually all of the data points were 100%.

Mycorrhizal colonization of *P. annua* was detected in every green sampled (Fig. 3), but the extent of colonization varied between courses. Levels of colonization were lowest on course C and highest on course B. Within a course, there were highly significant differences between greens in the extent of *P. annua* colonization (course A, $\chi^2 = 45{\cdot}06$; course B, $\chi^2 = 42{\cdot}56$; course C, $\chi^2 = 40{\cdot}02$; all $P < 0{\cdot}001$; all d.f. = 17).

On courses A and B, there was a significant negative relation between the colonization of *P. annua* roots by AM fungi and the abundance of this grass (Fig. 4). Therefore, in greens where colonization was high, the amount of *P. annua* in the sward was low. However, on course C, where colonization levels were very low (Fig. 3c), there was no significant relationship. Relationships between the extent of AM colonization of *A. stolonifera* and the abundance of this grass in the greens were also examined (data not shown). On courses A and C, there was a significant positive relation between these two parameters, indicating that in situations when the colonization of *A. stolonifera* was high, then this grass was more abundant, and vice versa. The significance of each relation was: course A, $F_{1,16} = 7{\cdot}9$, $P = 0{\cdot}012$; course C, $F_{1,16} = 20{\cdot}09$, $P < 0{\cdot}001$. Course B was not analysed because *A. stolonifera* only occurred in five greens (Fig. 2b). However, in no instance was there a correlation between the level of mycorrhizal colonization of *P. annua* and of *A. stolonifera*. Therefore, greens with high levels of colonization of *P. annua* did not also have high levels of *A. stolonifera* colonization. Furthermore, in no instance was there a correlation

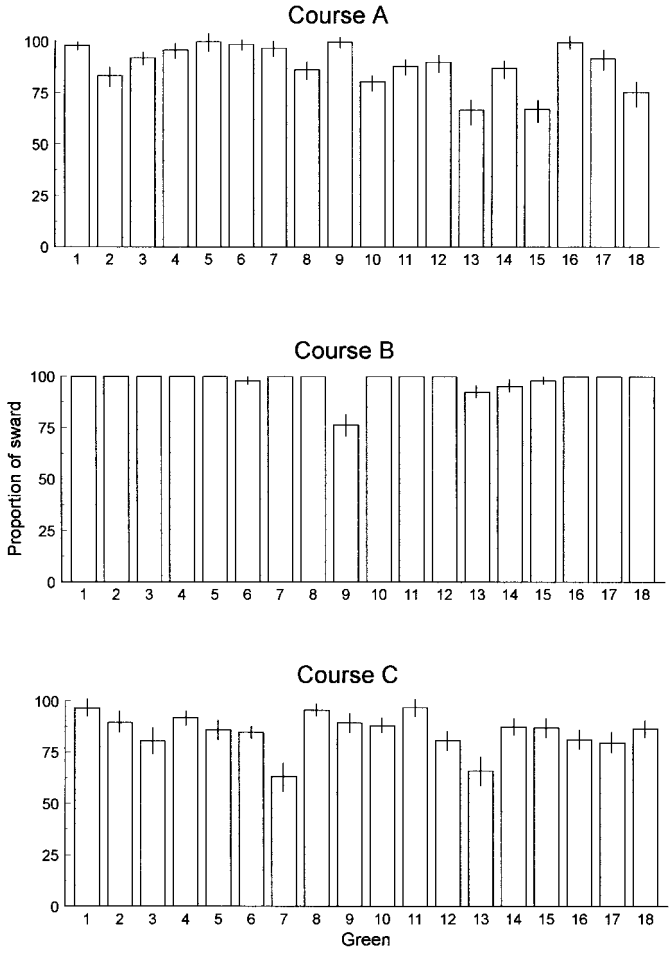

Fig. 2. Mean proportion of the sward formed by *P. annua* in each course. In 13 greens on course B all three replicates were 100%, hence there is no standard error.

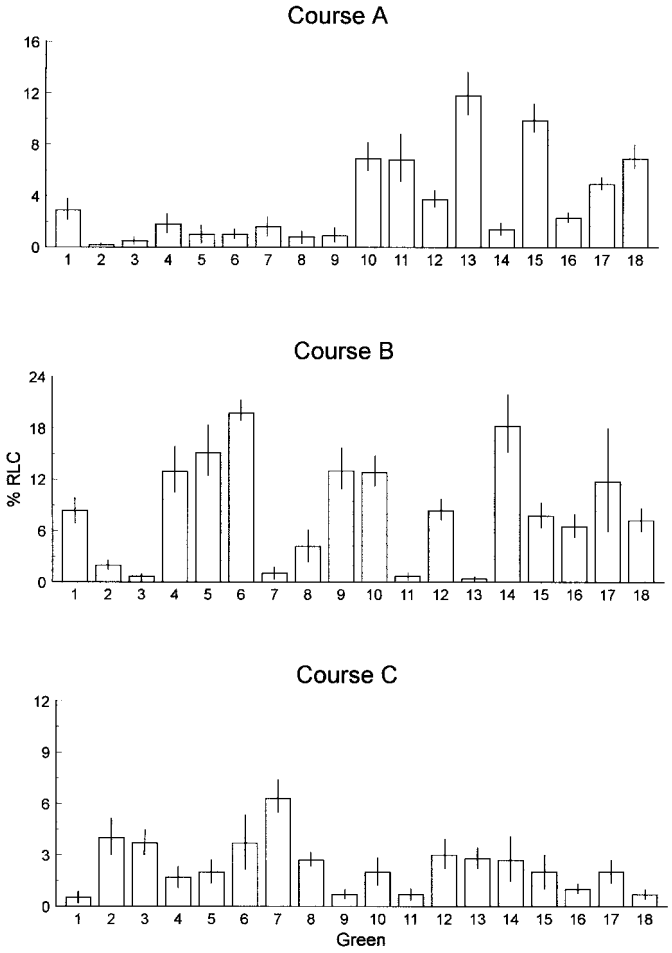

Fig. 3. Mean mycorrhizal levels of *P. annua* (expressed as percentage root length colonized; %RLC) in each course. Lines represent one standard error.

between the abundance of *P. annua* and *A. stolonifera*, suggesting that these grasses varied independently in their abundance.

MANIPULATIVE EXPERIMENT

The AM species composition of the green (percentage of all spores found) was: *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe, 40%; *G. fasciculatum* (Thaxt.) Gerd. & Trappe, 17%; *G. intraradices* N.C. Schenk & G. S. Sm., 14%; *G. versiforme* (P.A. Karst.) S.M. Berch, 8%; *G. etunicatum* W.N. Becker & Gerd., 7%; *G. caledonium* (Nicol. & Gerd.) Trappe & Gerd., 3%; and 11% of spores could not be identified.

Colonization levels of the three grasses in the green before and after the experiment are given in Table 1. It was encouraging that no significant differences in colonization levels were found in any treatment that did not receive fungal inoculum (control and seed addition treatments). Addition of mycorrhizas did not change the colonization levels of *P. annua* either, but those of *A. stolonifera* were increased in the inoculum and the inoculum plus seed addition treatments. Mycorrhizal colonization of *Festuca* spp. was increased by the addition of Vaminoc-T alone, but not when this was added with seed of *A. stolonifera*. Addition of mycorrhizal inoculum to a golf putting green can therefore result in increased colonization levels of the more strongly mycorrhizal grasses in the sward.

There were no significant differences between treatments in the abundance of any of the grass species at the start of the experiment. Total grass abundance in the four treatments is depicted in Fig. 5a. Addition of *A. stolonifera* seed had no significant effect on total abundance, but the addition of Vaminoc-T significantly elevated this measurement ($F_{1,20} = 24{\cdot}97$, $P < 0{\cdot}001$). This effect became most noticeable from mid-winter onwards when active growth began. The winter of 1997–98 was particularly mild in the study area. Although Vaminoc-T addition appeared to reduce the amount of *P. annua* in the sward (Fig. 5b), this was not statistically sig-

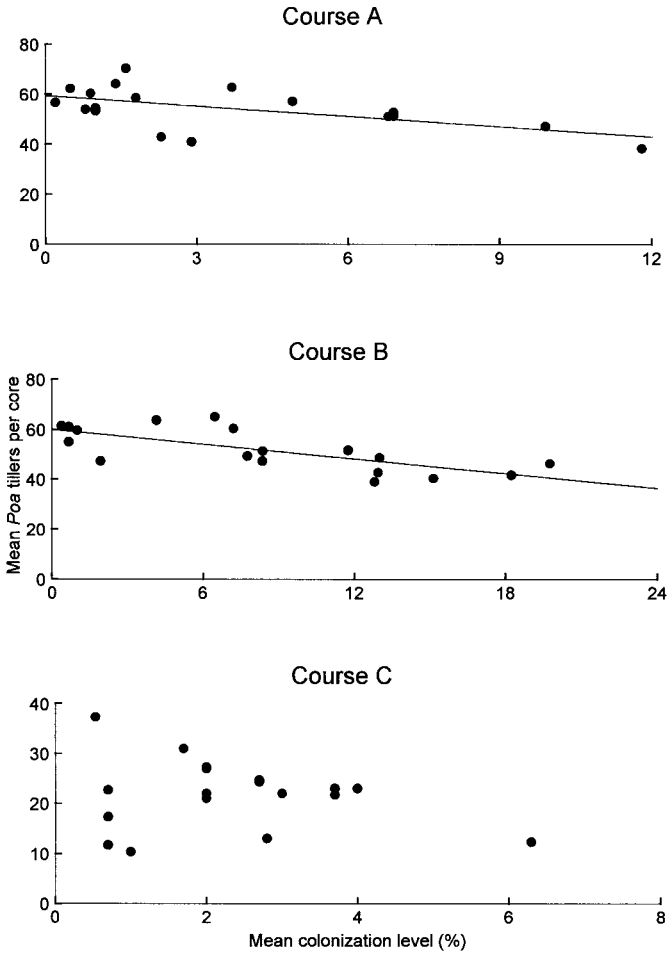

Fig. 4. Relations between the abundance of *P. annua* and mycorrhizal colonization of the roots. Significance of the relations: course A, $F_{1,16} = 7{\cdot}51$, $P < 0{\cdot}05$, $R^2 = 0{\cdot}32$; course B, $F_{1,16} = 17{\cdot}49$, $P < 0{\cdot}001$, $R^2 = 0{\cdot}52$; course C, $F_{1,16} = 0{\cdot}69$, $P > 0{\cdot}05$, $R^2 = 0{\cdot}04$.

nificant ($F_{1,20} = 1{\cdot}29$, $P > 0{\cdot}05$). However, there was a significant interaction between inoculum addition and date, indicating that the treatment effects differed over time. Thus, at the start of the experiment the inoculum-addition treatments had the highest amount of *P. annua*, but by the end these had less *P. annua* than the treatments that did not receive inoculum. There is therefore a strong suggestion that addition of Vaminoc-T to this green caused a reduction in the amount of *P. annua* in the sward, but this was not statistically proven.

A clearer response to mycorrhizal addition was evident in *A. stolonifera* (Fig. 5c). After December there was a clear separation of the treatments. Those plots in which mycorrhizas were applied had greater abundance of this grass than the control or

Table 1. Mycorrhizal colonization (%RLC) of the three grass species before (June 1997) and after (May 1998) the addition of mycorrhizal inoculum to a golf putting green. Values tabulated are means, with standard errors in parentheses. *A significant difference between June 1997 and May 1998 colonization levels at $P < 0{\cdot}05$ from Wilcoxon matched pairs signed rank test

	P. annua		*A. stolonifera*		*Festuca* spp.	
Treatment	June 1997	May 1998	June 1997	May 1998	June 1997	May 1998
Control	4·11 (0·51)	3·58 (0·96)	10·6 (1·86)	8·6 (2·65)	6·8 (2·88)	6·4 (3·57)
Seed addition	1·72 (0·93)	4·13 (0·87)	8·9 (1·69)	8·1 (1·85)	5·3 (2·69)	6·02 (2·88)
Vaminoc-T addition	3·91 (1·02)	3·15 (1·21)	9·8 (1·94)	16·2 (2·33)*	6·7 (2·36)	11·96 (2·68)*
Seed + Vaminoc-T	5·11 (1·13)	7·14 (1·84)	7·7 (1·37)	14·9 (1·96)*	7·7 (2·93)	8·25 (3·22)

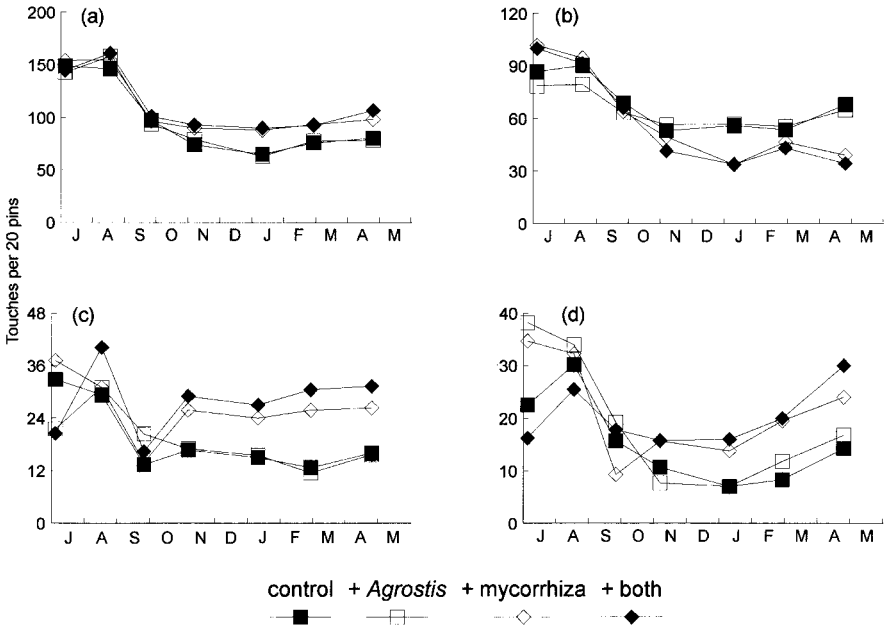

Fig. 5. Grass species abundance over the course of a year in the practice putting green with and without addition of *A. stolonifera* seed and mycorrhizal fungi. (a) All grass species; (b) *Poa annua*; (c) *Agrostis stolonifera*; (d) *Festuca* spp.

plots receiving only seed. The overall effect of mycorrhizal addition over time was significant ($F_{1,20} = 5{\cdot}59$, $P < 0{\cdot}05$) but the addition of the *Agrostis* seed had no effect on the number of tillers of this grass (Fig. 5c). Addition of mycorrhizal inoculum was therefore much more effective at increasing the abundance of *A. stolonifera* than the addition of seed of this grass species.

The pattern of abundance of *Festuca* spp. was similar to that of *A. stolonifera* (Fig. 5d), but the addition of inoculum had no significant effect on the abundance of this grass. By the end of the experiment, however, there was a clear suggestion that the *Festuca* spp. were more abundant in treatments where mycorrhizal fungi were applied, as opposed to where they were not. This was virtually the reverse of the situation at the start of the experiment.

Discussion

All the greens in this study were considered to be in good condition, indeed one of the courses was included in the list of the top 25 in the UK. However, it was obvious that the majority of the grass in each green was *P. annua*, rather than the more desirable bentgrass or fescue. Indeed, the latter was absent from virtually all the main greens in this study. The tiller counts were in line with recommendations for a good-quality playing surface (Perris & Evans 1996). Perhaps the greatest problem was the variable amount of *P. annua* in the greens on any one course. The result of this will be that the speed and accuracy of ball roll across the greens will vary; those with more *P. annua* will be less accurate and slower than greens that are predominantly bentgrass. Having different amounts of this grass in the sward will mean that the playing characteristics of each green will vary, but the management regime may also have to be varied to cope with the presence of this weed.

The fact that mycorrhizal colonization of *P. annua* was detected in all 54 of the greens is interesting and shows that this species does indeed form mycorrhizal associations, contrary to the statement by Hutchinson & Seymour (1982). Furthermore, the measurements in this study are of arbuscules only. We consider that it is more ecologically relevant to measure the colonization level of the definitive and functional part of the mycorrhiza, but if other structures such as vesicles and hyphae had been counted then the colonization levels would have been much higher. The levels of arbuscular colonization of *P. annua* found here were higher than total mycorrhizal levels found previously in a nearby natural plant community (Gange, Brown & Farmer 1990). The recording of AM species presence in each of the 54 greens was beyond the scope of this study. However, it is highly likely that differences in AM species composition exist between greens and between courses, as recorded by Koske, Gemma & Jackson (1997b). The structure of a plant community has recently been shown to be affected by the composition of the AM fungal community (van der

Heijden *et al.* 1998a,b). Therefore, AM fungal community differences may have led to the observed variation in abundance of *P. annua*. It must also be noted that our observed variation in root colonization of *P. annua* by AM fungi does not in itself indicate AM species variation, as fungal species effects are often independent of colonization levels (Streitwolf-Engel *et al.* 1997; van der Heijden *et al.* 1998a).

Similar to the previous study (Gange 1994), negative relations between the *P. annua* colonization level and abundance and positive ones between *A. stolonifera* colonization and abundance were found. The only exception to this was *P. annua* in course C, where AM colonization of this species was particularly low. We have two suggestions to explain these relations. The first is that the AM fungi are able to alter the balance of competition between the two grass species. Gemma *et al.* (1997a,b) have shown that AM fungi are highly beneficial to *A. palustris* and *A. canina*, in terms of their growth and drought resistance. It is highly likely that *A. stolonifera* exhibits a similar response. Meanwhile, *P. annua* is likely to be much less responsive (Gange, Brown & Farmer 1990). Several studies have shown that AM fungi can alter the balance of competition between grass species in favour of the most mycorrhizal species (reviewed in Newsham & Watkinson 1998). Such an explanation may account for the relations seen in course A. If *A. stolonifera* is more vigorous when fungi are common, one might expect this to also lead to the negative relation between *P. annua* and colonization levels. However, the fact that there were no relations between the abundance of the two grass species or between the colonization levels of the two species suggests that an alternative mechanism is also working to account for the negative interaction with *P. annua*. Furthermore, a competitive argument cannot explain the data from course B, where a significant negative relation with *P. annua* was found and yet *A. stolonifera* was only present in five of the 18 greens (Fig. 2b).

There have been a number of reports in the literature of AM fungal colonization of plants leading to reduced, rather than enhanced, growth (Johnson, Graham & Smith 1997) and this situation is not uncommon in grasses (Newsham & Watkinson 1998). Indeed, Smith & Read (1997) suggest that weakly mycorrhizal species may show negative responses to colonization, while more strongly mycorrhizal species show positive responses. If *P. annua* is in the former category, then the negative relation between colonization and abundance may be due to antagonistic effects of the fungi. Furthermore, negative effects of mycorrhizal colonization are often seen when P levels are high (Johnson, Graham & Smith 1997) and therefore the elevated soil P levels in putting greens may actually be an advantage in terms of mycorrhizas reducing the growth of this grass. However, it is likely that this will only be true up to a point; if P levels are exceptionally high then the fungi are likely to be affected adversely. Therefore, our working hypotheses for the relations between grass species abundance in golf putting greens and mycorrhizal fungi are that a competitive argument holds for *A. stolonifera* and an antagonistic one for *P. annua*. Our current experiments are aimed at establishing whether these ideas are true and, if so, the relative strengths of each mechanism.

These hypotheses have been generated from the observed differences in colonization levels of *P. annua* between greens and between courses. However the recent experiments of van der Heijden *et al.* (1998a,b) suggest that it is the species composition of the AM fungal community that is the driving force behind plant community structure. Nevertheless, the competitive and antagonistic arguments are still valid if the fungal species differ between greens. Clearly, the next objective must be to examine the responses of *P. annua* and *A. stolonifera* when colonized by different AM fungi, singly and in combination.

The management of AM fungal populations in the field can be achieved either by using a fumigant to reduce existing populations or adding inoculum to boost them. The latter approach has been plagued with problems, most notably the responsiveness of the crop, the species composition of the indigenous fungal population and the type of soil and its management (Smith & Read 1997). Furthermore, the difficulty with culturing the fungi means that inoculum is expensive to produce and rarely cost-effective. However, modern methods have improved production, and the inoculum used in the manipulative experiment here is now sold for use on sports turf. Given this fact, we now need to investigate the factors that could potentially hinder the efficacy of the inoculum. One example is again the species composition of the fungi in the greens. For example, it has been shown that one mycorrhizal species can inhibit colonization by another (Pearson, Abbott & Jasper 1993) and we may have been lucky in this experiment that no inhibitory fungi were present in the practice putting green. We have tested the four AM fungal species present in Vaminoc-T for their effects on the growth of *P. annua*. No positive effects have been recorded, while negative effects have been (A.C. Gange, unpublished data). It is therefore highly unlikely that addition of this inoculum could ever increase abundance of *P. annua* in fine turf, but the potential does exist for its reduction.

Addition of mycorrhizal inoculum resulted in enhanced levels of colonization in *A. stolonifera*. However, no change in colonization levels were seen

in *P. annua*. It is unfortunate that more frequent samples could not be taken from the green, but this would have damaged the putting surface beyond repair. One would always expect an amount of seasonal variation in colonization levels of roots, but it is encouraging that the only significant changes were found in treatments where inoculum was added, and all of these were increases. We can thus be reasonably sure that the changes observed were due to the treatment and not random environmental variation. Addition of inoculum to field soil is fraught with the difficulties described above, and therefore it is encouraging that this method of addition does seem to have the potential to increase mycorrhizal colonization in putting green soil. An increase in growth of *A. stolonifera* was seen in the treatments in which inoculum was applied and it may be no coincidence that this increase began to be seen clearly in winter, when abundance and growth of *P. annua* is at its lowest. As other species of bentgrass (*A. palustris* and *A. canina*) have been shown to have enhanced growth when colonized by *Glomus intraradices* Schenck & Smith (Gemma *et al.* 1997a,b) it is reasonable to assume that increased colonization of the *A. stolonifera* roots led to a concomitant increase in growth of this species. If the competition argument outlined above holds, then the corresponding decrease in *P. annua* may have been due to the mycorrhiza shifting the balance of competition between the grass species, in favour of *A. stolonifera*, a similar situation to that reported by Hetrick, Wilson & Hartnett (1989) and Hartnett *et al.* (1993). However, just because there was no measurable change in colonization levels of *P. annua* roots does not mean to say that particular fungal species colonizing the roots did not change, and so it is possible that the decrease in *P. annua* may also have been due to an antagonistic effect of the added fungi. Clearly we now need to perform analyses of fungal species composition in the roots of these grass species, similar to those reported by Clapp *et al.* (1995), in order to understand fully the dynamics of the mycorrhizal community in this system.

It is clear that addition of mycorrhizal fungi to a putting green can result in changes in the grass species composition of the green and that these changes are highly desirable to the greenkeeper. Furthermore, the addition of inoculum was considerably more effective in changing *A. stolonifera* abundance than was the traditional method of seed addition. Mycorrhizal addition appears to be able to promote overall grass growth, increase the growth of *A. stolonifera* and reduce that of *P. annua*. Once the mechanisms of these effects have been determined, then we should be able to improve the quality of putting green surfaces in addition to having a number of environmental benefits, such as reduced reliance on pesticides, water and fertilizer.

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